REMARKS

Reconsideration of the present application is respectfully requested in view of the above amendments and the following remarks. Claims 1, 17, 44 and 45 are currently pending and under examination. By the present Amendment, claim 1 is amended to more particularly point out and distinctly claim certain embodiments of the invention. No new matter has been added. Support for the amendments can be found in the specification and claims as originally filed; for example, on page 60, lines 24-33; page 61, lines 24-33; and page 62, lines 5-10. It should be noted that the above amendments are not to be construed as acquiescence with regard to the Examiner's rejections and are made without prejudice to prosecution of any subject matter removed or modified by this amendment in a related divisional, continuation or continuation-in-part application.

Rejections Under 35 U.S.C. § 103

A. The Examiner maintains the rejection of claim 1 under 35 U.S.C. 103(a) as being allegedly obvious over Holtzman (U.S. Patent No. 5,969,123) in view of Schatz (U.S. Patent No. 5,932,433) and further in view of Tall *et al.* (U.S. Patent No. 6,756,228). The Examiner asserts that Holtzman teaches a biochip comprising a biotinylated receptor protein immobilized via a biotinylation sequence motif, wherein the receptor protein has the ability of being specifically bound by a ligand of the receptor protein. The Examiner also asserts that Schatz teaches the biotinylation of a recombinantly expressed receptor protein within a bacterial host. The Examiner further asserts that Tall *et al.* teach a LOX-1 receptor immobilized to a substrate in order to detect the presence of LOX-1 activity. The Examiner asserts that it would have been obvious to include in the *in vivo* biotinylation as taught by Schatz in making the biochip of Holtzman, and that it would have been obvious to include as the receptor protein of Holtzman in view of Schatz, a receptor protein of LOX-1 as taught by Tall *et al.*

B. The Examiner rejects claims 17 and 44 under 35 U.S.C. 103(a) as being allegedly obvious over Brigham-Burke *et al.* (U.S. Patent No. 5,395,587) in view of Holtzman and further in view of Schatz and Tall *et al.* The Examiner asserts Brigham-Burke *et al.* teach a protein immobilized on a sensor chip substrate that conforms to a shape of an insertion site of a surface plasmon resonance device, and the Examiner further relies on Holtzman, Schatz and Tall *et al.*, as discussed above, to allegedly render the instant claims obvious.

C. The Examiner rejects claims 17 and 45 under 35 U.S.C. 103(a) as being obvious over Muramatsu (*Analytical Chemistry*, 1987;59:2760-2763) in view of Holtzman, and further in view of Schatz and Tall *et al.* The Examiner relies on Holtzman, Schatz and Tall *et al.*, as discussed above, and further asserts that Muramatsu teaches a protein immobilized on a crystal oscillator, which allegedly renders the instant claims obvious.

Applicants traverse these grounds for rejection and submit that the presently claimed subject matter satisfies the requirements of non-obviousness under 35 U.S.C. § 103(a). Applicants maintain that the instant claims are non-obvious for the reasons presented in the prior amendments, but provide the following additional comments specific to the current rejections.

With respect to the rejection outlined in section A above (and as also applieds to the rejections outlined in section B and C above), Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 1339 (Fed. Cir. 1997); the USPTO has the burden of showing a *prima facie* case of obviousness). The Examiner must at a minimum demonstrate that the combined references teach or suggest all the claim features, and even assuming, *arguendo,* that the combination of references teaches each claim feature, the Examiner must provide an explicit, apparent reason to combine these features in the fashion claimed by the Applicant with a reasonable expectation of success. *See KSR v. Teleflex, Inc.,* No. 04-1350 at 4, 14 (U.S. Apr. 30, 2007) ("A patent composed of several elements is not proved obvious merely by demonstrating that each element was, independently, known in the

prior art"). Here, the cited references, alone *or in combination*, fail to teach or suggest each feature of the instant claims, and in particular fail to teach or suggest a receptor chip comprising an immobilized scavenger receptor LOX-1, wherein the LOX-1 has the ability of being specifically bound by an endogenous ligand. In addition, the Examiner has not provided an explicit reason as to why one of skill in the art would have combined these references to achieve the presently claimed receptor chip with any reasonable expectation of success.

Applicants submit that the biotinylated LOX-1 receptor protein immobilized on the receptor chip, which is produced by refolding the biotinyated receptor protein expressed as an inclusion body in the host cell (as recited in claim 1), has the ability of being specifically bound by an endogenous LOX-1 ligand with an affinity close to that of LOX-1 protein expressed on a cell surface. This ability would not be expected based upon the understanding in the art at the time the instant application was filed, and none of the cited references describe a recombinantly produced LOX-1 receptor protein having this ability.

More specifically, Applicants submit that the present application provides data indicating that the non-glycosylated LOX-1 of the present invention binds to both AcLDL and OxLDL, typical endogenous ligands, and the dissociation constant (K_D) of the non-glycosylated LOX-1 of the present invention is 10^{-11} M according to BIACORE analysis (pages 60-62). Those skilled in the art would understand such an affinity to be very high (a K_D of 10^{-11} M indicates an affinity higher than that of a typical good antibody). This value is comparable to the dissociation constant of LOX-1 expressed on a cell surface. It is remarkably surprising that Applicants were able to produce an array comprising an immobilized LOX-1 protein able to bind to its endogenous ligands with such high affinity.

As described in detail in the prior response, it was previously known in the art that non-glycosylated LOX-1 has substantially reduced binding affinity. For example, Kataoka *et al.* (*Journ. Biol. Chem.*, 2000;275(9):6573-6579) teach that the affinity of non-glycosylated LOX-1 significantly decreases as compared to glycosylated LOX-1. Given this reduced affinity, the skilled artisan would not believe that a recombinantly produced LOX-1 polypeptide produced

according to the teachings of the cited references would necessarily have the ability to specifically bind an endogenous ligand, particularly when immobilized on a chip, as presently claimed. Accordingly, the prior art references fail to teach a recombinantly produced LOX-1 polypeptide that specifically binds to an endogenous ligand.

The Examiner asserts that it was not shown that the LOX-1 receptor protein described in Tall *et al.*, even if having reduced binding affinity to its natural ligand, could not be combined with the inventions of Holtzman and Schatz to arrive at the claimed invention. The Examiner further asserts that the claims do not specify to which ligand the receptor protein is capable of binding.

Without acquiescence to any basis of rejection, but to clarify the properties of the recombinantly-produced and non-glycosylated LOX-1 protein, Applicants have amended claim 1 to recite that the ligand the receptor protein is capable of binding is an endongenous ligand of the receptor protein. An example of a typical endogenous ligand includes OxLDL, which is described in Examples 3 and 4 of the original specification. Applicants submit that this amendment addresses the Examiner's concerns, and note that none of the cited references demonstrate substantial binding of a non-glycosylated recombinantly-produced LOX-1 protein to endogenous ligands.

Applicants further submit that even if the cited references could be combined to teach a receptor chip containing a recombinantly produced biotinylated LOX-1 protein, as asserted by the Examiner, this does not render the claimed invention obvious, since these references would not motivate the skilled artisan to combine their individual teachings to produce the claimed receptor chip. In addition, the Examiner has not provided an explicit reason as to why one of skill in the art would have combined these references to achieve the presently claimed receptor chip with any reasonable expectation of success. This lack of motivation and reason to combine the teachings of the various references is particularly striking, since the results obtained by Applicants would certainly not have been predictable to one of ordinary skill in the art.

As previously made of record the understanding in the art at the time of filing was that there were significant technical hurdles associated with expressing and purifying post-translationally modified mammalian receptor proteins, such as LOX-1, in high amounts using a bacterial host (*see, e.g.,* page 3, lines 8-26 of the specification). The cited references do not even remotely suggest that LOX-1 receptor protein can be successfully adapted to *in vivo* biotinylation and expression protocols. Producing functional, re-folded proteins that can be identically oriented due to *in vivo* biotinylation in an amount sufficient to produce receptor chips was not previously attainable prior to the disclosure of the present application. A person of ordinary skill in the relevant art would, therefore, have no reasonable expectation of success in arriving at the claimed subject matter based upon the teachings of the prior art references, as these references fail to teach or in any way suggest producing a ligand binding, correctly re-folded, *in vivo* biotinylated LOX-1 receptor protein from inclusion bodies in a bacterial host. Without specific guidance in this regard, mere mention of mammalian LOX-1 immobilized on a solid surface can not render obvious the presently claimed subject matter.

As evidenced by the present application and previously submitted, a person of ordinary skill in the art at the time of filing would not have reasonably expected biotinylated, mammalian LOX-1 produced from inclusion bodies in bacteria to correctly re-fold and bind its ligand. Instead, Applicants' substantial inventive efforts unexpectedly succeeded in adapting *in vivo* biotinylation and expression protocols for use with a highly post-translationally modified mammalian receptor protein, allowing LOX-1 to be produced for functional solid phase immobilization at levels higher than previously found in the art. Applicants therefore submit that the presently claimed receptor chip is non-obvious in view of Holtzman, Schatz, and Tall *et al.*, alone or in combination.

Furthermore, even if the Examiner had established a *prima facie* case of obviousness, the presently claimed receptor chip offers unexpected advantages over the teachings of the prior art, which further evidence the non-obvious nature of the presently claimed receptor chip. In particular, the receptor chip of the instant application attains significant effects in terms of detection of extremely low concentrations of endogenous ligand, thereby achieving advantages in terms of sensitivity that would not be expected by those skilled in the art of a

receptor described in Tall *et al.* was biotinylated *in vivo*, using the method described in Schatz, and employed in the invention of Holtzman.

In view of the expectation by one skilled in the art of significantly reduced binding, or even non-optimal binding, as acknowledged by the Examiner, of non-glycosylated LOX-1 to its natural ligand, the effects demonstrated by Examples 3 and 4 and Figures 3 and 4 of the instant application could not have been predictable to one of ordinary skill in the art in view of the cited references, and as such, the invention of claim 1 cannot be considered as being obvious. Particularly in light of Kataoka *et al.*, the ability of the claimed receptor polypeptides, produced as recited in claim 1, to specifically bind an endogenous ligand is clearly an unexpected result that would overcome a *prima facie* case of obviousness (M.P.E.P. § 2144.09 VII).

With regard to the rejections outlined in sections B and C above, Applicants also submit that the Examiner has failed to establish a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 1339 (Fed. Cir. 1997); the USPTO has the burden of showing a *prima facie* case of obviousness). As described above, Holtzman, Schatz and Tall *et al.*, alone or in combination, fail to teach the unexpected high affinity of non-glycosylated LOX-1 to endongenous ligands, such as AcLDL and OxLDL, of independent claim 1 as amended. In addition, the skilled artisan would have had no reasonable expectation of successfully producing a receptor chip comprising a LOX-1 protein having this ability. Thus, Applicants submit that the present amendments and above remarks also overcome the rejections of dependent claims 17, 44, and 45. In particular, neither Brigham-Burke *et al.* nor Muramatsu remedy the deficiencies as noted herein, as these references concededly fail to teach a biotinylated protein that is capable of binding its endogenous ligand with high affinity.

In view of the Remarks and Amendments provided herein, Applicants submit that claim 1 and those claims dependent therefrom satisfy the requirements of non-obviousness under 35 U.S.C. § 103 and respectfully request reconsideration and withdrawal of the Examiner's rejection.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Applicants respectfully submit that all of the claims remaining in the application are clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

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